## Claims

**What is claimed is:**

1. A method for producing a biological substance, comprising:

(a) cultivating a fungal host cell in a medium conducive for the production of the biological substance, wherein the fungal host cell comprises a first nucleic acid sequence encoding the biological substance operably linked to a second nucleic acid sequence comprising a promoter variant selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12; and subsequences thereof; and hybrid and tandem promoters thereof; and

(b) isolating the biological substance from the cultivation medium.

2. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 2, or a subsequence thereof; or a hybrid or tandem promoter thereof.

3. The method of claim 2, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 2.

4. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 3, or a subsequence thereof; or a hybrid or tandem promoter thereof.

5. The method of claim 4, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 3.

6. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 4, or a subsequence thereof; or a hybrid or tandem promoter thereof.

7. The method of claim 6, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 4.

8. The method of claim 1, wherein the promoter variant comprises the nucleic acid

sequence of SEQ ID NO: 5, or a subsequence thereof; or a hybrid or tandem promoter thereof.

9. The method of claim 8, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 5.

10. The method of claim 1, wherein the promoter variant comprises at least two copies of the sequence CGGCGTAATTTCGGCC.

11. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 6, or a subsequence thereof; or a hybrid or tandem promoter thereof.

12. The method of claim 11, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 6.

13. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 7, or a subsequence thereof; or a hybrid or tandem promoter thereof.

14. The method of claim 13, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 7.

15. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 8, or a subsequence thereof; or a hybrid or tandem promoter thereof.

16. The method of claim 15, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 8.

17. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 9, or a subsequence thereof; or a hybrid or tandem promoter thereof.

18. The method of claim 17, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 9.

19. The method of claim 1, wherein the promoter variant comprises the nucleic acid

sequence of SEQ ID NO: 10, or a subsequence thereof; or a hybrid or tandem promoter thereof.

20. The method of claim 19, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 10.

21. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 11, or a subsequence thereof; or a hybrid or tandem promoter thereof.

22. The method of claim 21, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 11.

23. The method of claim 1, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 12, or a subsequence thereof; or a hybrid or tandem promoter thereof.

24. The method of claim 23, wherein the promoter variant comprises the nucleic acid sequence of SEQ ID NO: 12.

25. The method of claim 1, wherein the promoter variant increases expression of the first nucleic acid sequence and is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5; and subsequences thereof.

26. The method of claim 1, wherein the promoter variant decreases expression of the first nucleic acid sequence and is selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12; and subsequences thereof.

27. The method of claim 1, wherein the hybrid promoter is comprised of portions of two or more promoters.

28. The method of claim 27, wherein the hybrid promoter comprises one or more portions

selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

29. The method of claim 28, wherein the hybrid promoter further comprises a portion of another promoter.

30. The method of claim 1, wherein the tandem promoter is comprised of two or more promoters.

31. The method of claim 30, wherein the tandem promoter comprises two or more promoters selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

32. The method of claim 31, wherein the tandem promoter further comprises another promoter.

33. The method of claim 30, wherein the two or more promoters of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence.

34. The method of claim 30, wherein one or more of the two or more promoters of the tandem promoter promote the transcription of the first nucleic acid sequence at different stages of growth of the fungal host cell.

35. The method of claim 1, wherein the fungal host cell contains one or more copies of the first nucleic acid sequence.

36. The method of claim 1, wherein the fungal host cell contains one copy of the first nucleic acid sequence.

37. The method of claim 1, wherein the biological substance encoded by the first nucleic acid sequence is a biopolymer.

38. The method of claim 37, wherein the biopolymer is selected from the group consisting of a nucleic acid, polyamide, polyamine, polyol, polypeptide, and polysaccharide.

39. The method of claim 38, wherein the polypeptide is selected from the group consisting of an antigen, enzyme, growth factor, hormone, immunodilator, neurotransmitter, receptor, reporter protein, structural protein, and transcription factor.

40. The method of claim 38, wherein the polypeptide is collagen or gelatin.

41. The method of claim 39, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

42. The method of claim 38, wherein the polysaccharide is chitin, heparin, hyaluronan, or hyaluronic acid.

43. The method of claim 1, wherein the biological substance encoded by the first nucleic acid sequence is a metabolite.

44. The method of claim 1, wherein the first nucleic acid sequence comprises a biosynthetic or metabolic pathway.

45. The method of claim 1, wherein the biological substance is native or foreign to the fungal host cell.

46. The method of claim 1, wherein the first nucleic acid sequence is contained in the chromosome of the fungal host cell.

47. The method of claim 1, wherein the first nucleic acid sequence is contained on an extrachromosomal element.

48. The method of claim 1, wherein the fungal host cell is a filamentous fungal or yeast cell.

49. The method of claim 48, wherein the filamentous fungal cell is an *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliophthora*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium*, or *Trichoderma* cell.

50. The method of claim 48, wherein the yeast cell is a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* cell.

51. The method of claim 48, wherein the filamentous fungal host cell is an *Aspergillus* cell.

52. The method of claim 48, wherein the filamentous fungal host cell is a *Fusarium* cell.

53. An isolated promoter variant comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12; and subsequences thereof; and hybrid and tandem promoters thereof.

54. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 2 or a subsequence thereof; or a hybrid or tandem promoter thereof.

55. The promoter variant of claim 54, which comprises the nucleic acid sequence of SEQ ID NO: 2.

56. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 3 or a subsequence thereof; or a hybrid or tandem promoter sequence thereof.

57. The promoter variant of claim 56, which comprises the nucleic acid sequence of SEQ ID NO: 3.

58. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 4 or a subsequence thereof; or a hybrid or tandem promoter sequence thereof.

59. The promoter variant of claim 58, which comprises the nucleic acid sequence of SEQ ID NO: 4.

60. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 5 or a subsequence thereof; or a hybrid or tandem promoter thereof.

61. The promoter variant of claim 60, which comprises the nucleic acid sequence of SEQ ID NO: 5.

62. The promoter variant of claim 53, wherein the promoter variant comprises at least two copies of the sequence CGGCGTAATTTCGGCC.

63. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 6 or a subsequence thereof; or a hybrid or tandem promoter thereof.

64. The promoter variant of claim 63, which comprises the nucleic acid sequence of SEQ ID NO: 6.

65. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 7 or a subsequence thereof; or a hybrid or tandem promoter thereof.

66. The promoter variant of claim 65, which comprises the nucleic acid sequence of SEQ ID NO: 7.

67. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 8 or a subsequence thereof; or a hybrid or tandem promoter thereof.

68. The promoter variant of claim 67, which comprises the nucleic acid sequence of SEQ ID NO: 8.

69. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 9 or a subsequence thereof; or a hybrid or tandem promoter thereof.

70. The promoter variant of claim 69, which comprises the nucleic acid sequence of SEQ ID NO: 9.

71. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 10 or a subsequence thereof; or a hybrid or tandem promoter thereof.

72. The promoter variant of claim 71, which comprises the nucleic acid sequence of SEQ ID NO: 10.

73. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 11 or a subsequence thereof; or a hybrid or tandem promoter thereof.

74. The promoter variant of claim 73, which comprises the nucleic acid sequence of SEQ ID NO: 11.

75. The promoter variant of claim 53, which comprises the nucleic acid sequence of SEQ ID NO: 12 or a subsequence thereof; or a hybrid or tandem promoter thereof.

76. The promoter variant of claim 75, which comprises the nucleic acid sequence of SEQ ID NO: 12.

77. The promoter variant of claim 53, which increases expression of the first nucleic acid sequence wherein the promoter variant is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5; and subsequences thereof.

78. The promoter variant of claim 53, which decreases expression of the first nucleic acid sequence, wherein the promoter variant is selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12; and subsequences thereof.

79. The promoter variant of claim 53, wherein the hybrid promoter is comprised of portions of two or more promoters.

80. The promoter variant of claim 79, wherein the hybrid promoter comprises one or more portions selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,

SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

81. The promoter variant of claim 80, wherein the hybrid promoter further comprises a portion of another promoter.

82. The promoter variant of claim 53, wherein the tandem promoter is comprised of two or more promoters.

83. The promoter variant of claim 82, wherein the tandem promoter comprises two or more promoters selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

84. The promoter variant of claim 83, wherein the tandem promoter further comprises another promoter.

85. The promoter variant of claim 82, wherein the two or more promoters of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence.

86. The promoter variant of claim 82, wherein one or more of the two or more promoters of the tandem promoter promote the transcription of the first nucleic acid sequence at different stages of growth of the fungal host cell.

87. A nucleic acid construct comprising a nucleic acid sequence encoding a biological substance operably linked to the promoter variant of claim 53; or subsequences thereof; or hybrid and tandem promoters thereof.

88. A recombinant expression vector comprising the nucleic acid construct of claim 87.

89. A recombinant host cell comprising the nucleic acid construct of claim 87.

90. A method for producing a biological substance, comprising (a) cultivating a

homologously recombinant cell, having incorporated therein a new transcription unit comprising a promoter variant of claim 53, an exon, and/or a splice donor site operably linked to a second exon of an endogenous nucleic acid sequence encoding the biological substance, under conditions conducive for production of the biological substance; and (b) recovering the biological substance.